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Anatomically preserved Silurian 'nematophytes' from the Welsh Borderland UK.

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ABSTRACT: Stratified charcoalified fragments of thalloid organisms with tripartite tissue construction have been isolated from the basal member of the Upper Silurian (upper Ludlow) Downton Castle Sandstone Formation, exposed near Ludlow, Shropshire, England and are considered to have had fungal affinity. They are divided into two major groups. The more novel is characterised by a superficial cortex separated from a basal layer of interweaving hyphae by an intermediate zone of compressed indeterminate tissue and members are placed in a new taxon, *Tristratothallus ludfordensis*. In the second, the intermediate zone comprises hyphae arranged at right angles to the cortex (termed palisade). Some members resemble the tissue construction of *Nematothallus* Lang described from the Lower Devonian (Lochkovian) of the Welsh Borderland and considered to belong to fungi, some of which were lichenised. A further type, which shows remains of polysporic asci, is

thought to represent a fragment of an apothecium (a disc-shaped ascoma of an ascomycete) of a Pezizomycete and is the earliest such record. Yet others are characterised by a perforate cortex with occasional protruding hyphae, whose tissue construction was also recorded in the Lower Devonian of the Welsh Borderland and considered to display fungal characteristics. Coalified 'black patches' are common on bedding surfaces throughout the latest Silurian and early Devonian and frequently are associated with basal embryophytes and tracheophytes. Those reported here are the oldest known with three dimensional organisation, studied via scanning electron microscopy (SEM) and attributed to the fungi, and include some ascomycetes. Similar encrustations occur in even earlier rocks and may have been important constituents of the cryptogamic ground cover, which is postulated to have preceded higher plant life on land.

KEY WORDS: Ascomycetes – *Cosmochlaina* – fungi – *Nematothallus* – Pezizomycetes.

INTRODUCTION

Recent interest in non-vascular plant components of early terrestrial vegetation has focussed on cryptogamic ground covers as a modern analogue of ancient communities (Tomescu & Rothwell 2006) and in particular the role of nematophytes (Edwards & Kenrick, 2015; Edwards, Cherns & Raven, 2015), here considered to possess fungal affinities (Edwards, Axe & Honegger, 2013), in their composition. It has been shown that in the Lower Devonian dispersed 'cuticles' assigned to *Nematothallus* (Lang, 1937) and *Cosmochlaina* (Edwards, 1986) were superficial remains of a tripartite stratified thallus, some of which were identified as lichenised fungi (Honegger, Edwards & Axe, 2013). All these three dimensionally preserved examples were isolated from Lower Devonian rocks in the Welsh Borderland, but

similar ‘cuticles’ have been isolated from older Silurian strata in the area. Here we report on fragmentary stratified organisms with hyphal construction and again possessing three tissue types from rocks exposed above the famous Ludlow Bone Bed at Ludford Corner, Ludlow, England. These strata were originally considered of Přídolí age, but more recently were assigned to the mid Ludfordian (late Ludlow) (Loydell & Fryda, 2011). The methods of isolation and subsequent analysis using scanning electron microscopy were those utilised in the Lower Devonian investigation (Edwards, Axe & Honegger, 2013). Some examples are very similar to the younger specimens in the presence of an intermediate ‘palisade’ zone (Group 2 below), but a number lack this and will be placed in a new genus, *Tristratothallus*, in the Nematothallaceae (Strother, 1993). The overall construction of the two types is given in Figure 1. While the majority are considered vegetative fragments of a thalloid organism, some are tentatively interpreted as fragments of a fungal hymenium.

SYSTEMATICS

ORDER: NEMATOPHYTALES LANG 1937

FAMILY: NEMATOTHALLACEAE STROTHER 1993

Type genus: Nematothallus Lang 1937

Tristratothallus Edwards, Axe, Honegger & Morris **gen. nov.**

Diagnosis: Fungus with stratified thalloid organisation comprising three tissue types; a unistratose superficial cortex, a basal layer of interweaving hyphae and indeterminate intermediate zone.

Derivation of name: *Tristratus* (L) = three-layered. *Thallus* (L) = body of a fungus.

Type species: *Tristratothallus ludfordensis*.

Tristratothallus ludfordensis Edwards, Axe, Honegger & Morris **sp. nov.**

Diagnosis: As for genus.

Derivation of name: From the locality, Ludford Lane, Ludlow, Shropshire, England.

Holotype: LL(L)07/03; turned over on LL(L)19/01.

Illustrations: Fig. 2B, E-G; Fig. 3B, D, H, L.

Locality: Road-side cutting in Ludford Lane around the corner from the famous Ludlow Bone Bed locality, Ludford Corner, south of Ludlow, Shropshire, England.

Stratigraphy: Fossiliferous horizon occurs in the Ludlow Bone Bed Member (LBBM), 0.15-0.20 m above the Ludlow Bone Bed. As the basal member of the Downton Castle Sandstone Formation, the LBBM, originally considered the base of the Přídolí Series, is now considered mid Ludfordian (late Ludlow) in the Silurian System.

DESCRIPTIONS

Group 1: Palisade absent (LL(L)07/03; LL(L)07/17; LL(L)07/13; LL(L)07/06). *Characterised by a superficial cortex, separated from a basal layer of interweaving hyphae by an intermediate zone of compressed indeterminate tissue, exemplified by* Tristratothallus ludfordensis *gen. et sp. nov.*

i) *Tristratothallus ludfordensis* gen. et sp. nov.

Description: The description is based on four small fragments, all of which show a tripartite stratified organisation. The largest measured 1.87 by 1.16mm, with thicknesses varying between 55 and 120µm (Fig. 2, A, B). The uppermost and basal layers are best preserved. The former comprises a single layer of usually incomplete cells (Fig. 2, B, G, H) here termed a cortex as defined in earlier work on stratified nematophytes (Edwards *et al*., 2013). In surface view the cells are slightly domed with usually no indication of separation of individual cells (Figs. 2C, D; 3A). They are

more or less isodiametric and range between 5 and 11µm in diameter. Small areas show dark lines between the cells (Fig. 2F). In others there are central depressions where the wall may be absent (Fig. 3C). In vertical fractured section, the inner periclinal wall is usually missing: anticlinal walls may curve inwards in this area (Fig. 2H). The latter are best seen in tangential fractured section (Figs. 2D, E; 3B). Inner periclinal walls occur in the best preserved specimen. The basal layer comprises interweaving tubular structures interpreted as hyphae. Seen from below (Fig. 3H-K), the intact hyphae show variation in diameter, but with a remarkably consistent width between specimens (c. 5-6µm mean). Most are parallel-sided, straight or occasionally sinuous and unbranched (Fig. 3J, K). Septa have not been observed. In some instances, intra-specimen variation in width may relate to thickness of hyphal walls, the narrowest frequently being twisted and distorted. Such variation is visible on vertically fractured surfaces (Fig. 3L-P). Branching, although rare, ranges between isotomous, lateral and clustered, although there is the possibility that such organisation relates to homogenisation of superimposed hyphae (Fig. 3H). In three examples, this basal layer is compact, probably due to some taphonomic compression, but the fourth shows fragmentary hyphae perpendicular to the surface, perhaps a better indication of organisation in life where this zone would have been in contact with a substrate (Fig. 3Q). The region sandwiched between the cortex and hyphal basal layer is difficult to interpret as in vertical fractured section it comprises highly compacted, fused coalified material with some irregular voids (Fig. 2A, B). Fractured tangential sections (Fig. 2C, E) provide no information on three dimensional organisation, although there are hints at a laminate construction. Most of the fragmentary sheets have smooth walls, but all specimens show examples with small circular, elliptical or irregularly shaped (but with rounded edges) perforations or depressions (c. 1µm maximum diameter) (Fig. 3D-G).

The type of preservation of this intermediate zone suggests that it was thicker in life, possibly with interhyphal air spaces such that collapse occurred on compression. It seems likely (from the three dimensional nature of the remaining layers) that the latter was not excessive.

Comparison with Lower Devonian thalli with tripartite organisation: The majority of the thalli described by Edwards *et al*. (2013) possessed three layers but these Silurian examples lack the intermediate zone in which hyphae were orientated at right angles to the surface. They also differ in that no specimens show the separation of the outer periclinal walls of the cortex that produced the *Nematothallus* isolated cuticles, although the organisation of the cortical cells would produce a similar patterning. A younger exception as regards palisade hyphae is the specimen described under *Cosmochlaina* Type 5 (NMW 2013.39G.21), so placed because the surface possessed occasional perforations (Edwards *et al*., 2013, fig. 6B). It differed from the remaining Lower Devonian specimens because the cortex was three layered and the tissue below, although described as palisade, was not composed of well-defined hyphae. Instead the zone showed comparable gross organisation perpendicular to the surface, but is composed, along with strands, of amorphous sheets with small holes sometimes associated with irregular cavities (Edwards *et al*. 2013, fig. 6C-D). The holes are of similar size, but less frequent than those in the Silurian examples. Indeed on compression such sheets might have produced the coalified material noted in the older example. They were interpreted as sheaths possibly of cyanobacterial colonies intermingled with poorly preserved hyphae, thus allowing the inference of a possible lichen affinity. However, the specimen was quite different in organisation from the stratified lichenised thalli noted from the same Lower Devonian locality (Honegger *et al*., 2013). In view of such differences we have erected a new genus.

The presence of a well-developed basal layer of thin-walled hyphae is considered evidence for a substrate encrusting thallus in life.

ii) cf. *Tristratothallus* (Specimen LL(L)06/16).

Description: Fragment with irregular outline, 800 x 250µm in area, comprising superficial layer and traces of underlying tissues, but no intermediate palisade zone of well-preserved hyphae (Fig.4A) or a basal layer. The surface is variable in appearance but dominated by prominent, hollow, contiguous projections of differing shapes (Fig. 3C, H) and sizes (4.6 - 10.9µm in diameter, n=40, x=7.2µm). Smaller areas are much flatter with similarly sized, gently elevated, structures sometimes in continuity, but more frequently separated by perforated lines (Fig. 4D). In places, the layer of projections has peeled away, revealing a reticulum of ridges below (cf. *Nematothallus*) (Fig. 4B, F, I) and more deeply, discontinuous, irregular, masses of material (Fig. 4G) or similar material with occasional remnants of hyphae (Fig. 4B, E). In small areas there is evidence for inner periclinal walls that confirm the original presence of a cortex (Fig. 4J). In the coalified remains beneath the cortex, there is just one area with minute perforations in a flat surface similar to those described in *Tristratothallus* (Fig. 4H), and homogenisation of underlying tissues is seen in occasional fractured vertical sections through the specimen (Fig. 4J).

Comparisons and interpretation: While such similarities are shared with *Tristratothallus* (viz. homogenisation of underlying tissues and microperforate sheets), the specimen differs in the nature of the superficial layer because the projections are more developed (taller). There are certain lichens where globose cells of a cortex grow into filamentous hair-like hyphae forming a water-absorbing tomentum (e.g. *Sticta* (Schreb.) Ach., *Teloschistes* Norman; Honegger, 2009) or a means of capturing cyanobacteria (e.g. certain *Peltigera* spp. Willd.; Honegger,

2012).

Group 2: Palisade present.

Characterised by specimens with three layered organisation comprising a cortex and in the thickest fragments, an intermediate zone of hyphae orientated at right angles to the cortex and a basal layer of wefts of smaller hyphae.

Variation in superficial tissues suggests that a number of taxa are present, hence the separate treatment of a number of specimens. Variation might also reflect different developmental stages of a reproducing organism, probably of fungal affiliation.

Group 2A: Cortex imperforate.

i) *Nematothallus* sp. (Specimens LL(L)04/02, LL(L)04/13, LL(L)07/02, LL(L)16/05; Figure 5).

The specimens in this group show a range in preservation in the three layers preventing confident diagnosis at species level. All specimens are fragmentary but a significant number show marginal curvature both in gross outline and inrolling of tissue suggestive of the limits of the organism. Specimen LL(L)04/13 is an example with a more or less circular outline with centrally located basal layer of hyphae that might reflect original shape of a very small organism, although erosion during transport is a further possibility (Fig. 5A). It possesses a unistratose cortex in which the thin inner periclinal walls are occasionally preserved. Underlying tissues of the intermediate zone in all specimens consist of poorly preserved hyphae, orientated at right angles to the cortex and basal layer (Figs. 5C, D, F, H), the latter forming hyphae are barely discernible but better evidence for their nature is seen in Figure 5G, where narrow, very fragmented, hyphae are visible to the inside of the fused limiting layer. Such organisation is present in specimen LL(L)16/05, where clearly

delimited cells with more resilient, slightly convex, outermost walls form a continuous uninterrupted cortex (Fig. 5B). The nature of the transition between intermediate palisade zone and both cortex and basal layer cannot be distinguished in any specimens. Variation in the thickness of the often granular disorganised tissue above the palisade zone may reflect the thickness of the original cortex (defined in *Nematothallus* as at least one layer thick), be it uni- or multistratose. Figure 5E shows such a typically extremely poorly preserved example of the group in which an inner reticulate surface, typical of dispersed cuticles, is evident plus sparse remnants of presumed palisade hyphae.

Comparisons and affinities: In 2013, Edwards *et al*. related dispersed cuticles named *Nematothallus* (e.g. Edwards, 1982) and *Cosmochlaina* (e.g. Edwards, 1986) in palynomorph assemblages to thalli with tripartite organization—uni- or multistratose cortex, an intermediate zone of palisade hyphae and a basal layer of wefts of narrow hyphae. The specimens here with imperforate/unornamented cortex are assigned to *Nematothallus*. The dearth of fragments that possess all three layers and/or allow useful measurements prevents further specific diagnosis, but limited data indicate that they fall within the range of *N. williamii* recorded from the Lochkovian (Edwards *et al.*, 2013) although secure assignment would require more detail of the palisade tissue. Relevant data are not available for the lectotype, *N. pseudo-vasculosa* from Přídolí strata in Pembrokeshire, south Wales, which was so designated by Strother (1993) from Lang's coalified compressions (Lang, 1937) and lacks evidence of a cortex.

ii) Ascomycete apothecial fragment (Specimen LL(L)03/02; Figure 6).

This small fragment, 770 x 545µm in surface area and up to 250µm thick (Fig. 6A), was first described and illustrated by Honegger *et al*. (in press) as comprising

apothecia containing polysporic asci of a possible Pezizomycete. Parallel thin-walled hyphae limit the apothecia (Fig. 6C, E) with individual asci (Fig. 6D) developing between sterile paraphyses, in a c.200µm thick hymenial zone (Fig. 6C, H). Here we provide further confirmation on the superficial layer and a third less well preserved basal layer. The presumed upper surface of this specimen is covered by continuous, but separated, globular to irregularly shaped, hollow outgrowths (Fig. 6B) of maximum diameter (4.9-8.9µm, x=6.7µm, n=25). The majority were interpreted by Honegger *et al*. (in press) as the tip cells of paraphyses, but evidence for continuity (Fig. 6H, I) with underlying tissues was equivocal. Areas of the basal layer of the fragment show wefts of hyphae (Fig. 6F, G) similar to those described in the Group 1 stratified thalli. These hyphae show variation along their lengths, possibly resulting from thin walls, and branching is more common. Mean diameter is 4µm and range 1-6µm.

Comparisons and discussion on affinities: This specimen represents the earliest yet known fragment of an apothecium (disk-shaped, sexual reproductive structure) of a Pezizomycete, comparable with the often quite large apothecia of extant representatives, e.g. in the genera *Peziza*, *Sepultaria* etc. It might have grown in a terrestrial soil crust community, presumably as a saprotroph, its vegetative mycelium having derived nutrients by degrading organic material within the soil. When nutrients are getting scarce such disk-shaped ascomata of extant taxa appear, often in large groups, on the soil surface. After spore release they die off and are soon degraded. Charcoalification most likely was the best mode of preservation of this presumably short-living fruit body.

iii) Putative apothecial fragment (Specimen LL(L)16/02, Figure 7).

This fragment is roughly oval with irregular margins and maximum dimensions,

c.780x 1000 mm. It is unusual in that there is a continuous, thick, almost amorphous sheet (Fig. 7A) above a ‘cellular ‘zone, superficially fractured to reveal anticlinal walls of a presumed hymenial surface layer (Fig. 7B), which in deeper tangential sections forms an irregular reticulum (Fig. 7C). This is separated from an intermediate zone of hyphae orientated at right angles to the presumed multistratose superficial tissue by a disorganised granular region that makes it impossible to discern the nature of the junction between the two layers. The intermediate zone comprises poorly preserved hyphae, sometimes fused to form sheets. There is variation in the packing of the hyphae: they may be closely spaced (Fig. 7D or widely separated forming cavities (Fig. 7G) which are occasionally filled with pyrite. The latter may represent the original position of asci, and suggests that the hyphae were paraphyses separated from the exterior by an extensive epihymenial layer. Asci were not preserved; as shown by Honegger *et al*. (in press) their wall structure and composition differs from the paraphyses. The basal layer is compressed with individual hyphae barely discernible (Fig. 7F), except at the surface where they may be contaminants (Fig. 7G).

Comparisons and discussion on affinities: The absence of direct evidence of ascospores and the complex organisation of the superficial tissue (?epihymenium) cautions against unequivocal identification of the fragment as part of a hymenial layer, but the organisation of the hyphae in the intermediate ‘palisade’ zone bears comparison with the organisation of the fragment attributed to the ascomycetes above.

iv) Putative apothecial fragment (Specimen LL(L)05/09, Figure 8).

This wedge-shaped fragment (Fig. 8A) has a maximum width of 365µm, tapering to a point and is 435µm long, with conspicuous hyphae orientated at right angles to the

surface. The latter is basically smooth with minute perforated pustules (Fig. 8E), but raised into irregular folds and craters which are typical products of charcoalification (Fig. 8C, E). There is no obvious cellular surface layer, but the raised reticulum on the inner surface of the limiting layer is similar that seen on the isolated 'cuticles' assigned to *Nematothallus* (Fig. 8B) and here interpreted as the impressions of the tips of paraphyses on the amorphous outer layer. The underlying tissues comprise well defined hyphae (8.5-13.6 µm, x=11.3µm, n=9) (Fig. 8A) with thick walls (1.5-2.6µm wide) accompanied by thinner-walled irregular strands of similar orientation (Fig. 8D, F) plus abundant indeterminate disorganised organic material: the entire (but proximally incomplete) zone at least 335µm deep (Fig. 8B). The prominent hyphae are 8-14µm long with rounded tips (Fig. 8A, G) possibly at both ends. A rare example of a tip shows a central roughened area (Fig. 8H). More proximally, individual hyphae may be deeply furrowed. There is also limited evidence for interconnections proximally. The precise relationship of these thick-walled hyphae and the similarly orientated thinner walled 'stringy' entities (Fig. 8B, D) with the superficial layer is unknown. There is no evidence for organic continuity. In some places where separation has occurred the underlying tissues are separated by granular material (Fig. 8D). The coalified remains between the hyphae are variable; sometimes sparse and irregular, elsewhere almost sheet-like (Fig. 8B). In places, elongate structures have irregular surfaces. The latter are somewhat similar to the remains interpreted as the contents of asci by Honegger *et al*. (accepted) (Fig. 8B, C).

Comparisons and affinities: Although a basal layer is absent, overall similarities suggest that this specimen was originally tripartite. Poor preservation of the superficial layer hampers further comparison but the absence of obvious perforations suggest it is closest to *Nematothallus* although the perpendicular (palisade) hyphae

appear far more robust with thicker walls and are narrower than those seen in the Lower Devonian *N. williamii* (means 11.3 v.14.8 µm). However, should asci indeed be present, the fragment would have derived from the hymenial layer of a polysporic ascomycete.

Group 2B: Characterised by occasional surface projections (cf. Cosmochlaina*) and intermediate forms* sensu *Edwards et al. 2013.*

i) Specimen LL(L)10/21 (Figure 9).

Roughly elliptical fragment with maximum dimensions c. 900 x 1150µm (Fig. 9A, B). Outer surface has a very irregular appearance due to presence of numerous, often contiguous, projections of varying shapes (Fig. 9C-H) with occasional intervening smoother areas (Fig. 9D, F). In some areas the close-set projections are separated by deep incisions (Fig. 9G), in others the surface is not interrupted resulting in less pronounced relief (Fig. 9C). On rare occasions a further circular outgrowth occurs within a typical globular projection (Fig. 9E, K) or the outer wall may be missing or broken down in this area (Fig. 9J). Many of the projections are more or less isodiametric, either globose or hemispherical with straighter sides where more crowded. Occasional examples appear fused (Fig. 9I), tongue-shaped (Fig. 9G) or even bifurcated (Fig. 9H).

Tissue preservation is poor below the superficial layer (Fig. 9L). There is indication of fused hyphae at right angles to the edge (Fig. 9L, O) with some intervening areas filled with granular material (Fig. 9M, N). Fractured surfaces indicate some layering, but again no well-defined hyphae, except for very small examples, which may be contaminants (Fig. 9P). The nature of the relationship between the superficial layer and the putative palisade zone cannot be elucidated (Fig. 9I, O).

Also present on the lower surface and partly overlying the hyphal zone are two

almost circular compressed granular areas, 820 and 645µm in diameter (arrowed in Fig. 9A).

Comparisons and affinities: Assuming that the specimen represents the remains of a highly compressed tripartite thallus, with intermediate palisade zone, the specimen is closest to specimen LL(L)03/02 (Fig. 6) which was considered a possible Pezizomycete by Honegger *et al*. (accepted). The granular structures interpreted as polysporic asci in Fig. 7D, E are similarly preserved. Those authors interpreted the 'palisade' hyphae as paraphyses whose free tips produced the surface projections that were intermingled with the tips of asci. Similar surface projections have been recorded in *Cosmochlaina*, where they are far more frequent (Edwards *et al*., 2013). Whether or not the different morphologies of the projections provide information on the development of the paraphyses tips remains conjectural. An alternative explanation is that they represent the further development/ maturation of a cortex. The apparent breakdown of wall noted in Figure 9J might be related to dispersal from an ascus. The compressed masses are comparable to the medullary spots noted in reproductive structures attributed to *Prototaxites* (Honegger *et al*. accepted), but better structural evidence is needed to confirm this.

ii) Specimens LL(L)04/06 and LL(L)11/11 (Figure 10).

Less well preserved than the previous example, in specimen LL(L)04/06 (Fig. 10A) the surface of the cortex is irregular marked by both depressions (Fig. 10B, C) and areas of irregular outgrowths similar to those illustrated in Figure 9D-G including occasional papillae/ protruding hyphae. Palisade hyphae are present (Fig. 10G, H), but poorly preserved, while basal layer of hyphal wefts show variation in hyphal diameter (3-4.5-7 µm, n=21) (Fig. 10F) and branching. Similar dimpling of the upper surface is seen on the inrolled margin of specimen LL(L)11/11 with ?incipient

projections (Fig. 10L, arrowed) revealed in oblique fracture of the surface (Fig. 10I, J) and deeper tangential sections (Fig. 10K). Protruding hyphae also occur on disintegrating upper surfaces (Fig. 10E). The two remaining zones are present but less well preserved (Fig. 10H), the basal one almost completely homogenised but with probable contaminants (Fig. 10M).

iii) *Specimens with surface incisions* (LL(L)10/23, LL(L)11/09, LL(L)02/07 (Fig. 11). These are best exemplified by specimen LL(L)10/23 (Fig.11A) where more or less evenly spaced mostly rounded 'units' incised to varying degrees on a thick 'cuticle', there being no internal tissues preserved. Similar layers overlie palisade hyphae in other specimens (Fig. 11B). The best preserved (LL(L)02/07) shows expanded hyphae below the cortex (Fig. 11C, D) and some superficial disintegration in the incised areas (Fig. 11E), characters shared with specimens with occasional projections that typify intermediate forms *sensu* Edwards *et al*. (2013). Others reveal little hyphal detail in either palisade or basal regions, which show some evidence of colonisation by filamentous bacteria (Fig.11F).

Comparisons and affinities: Thalli assigned to *Comochlaina* differ from those of *Nematothallus* in that the cortex surface bore projections sometimes accompanied by perforations. Edwards *et al.* (2013) recognised at least five types in the Lochkovian based on surface variation. Specimens with similar features occur in the Silurian specimen, which is particularly close to Lochkovian specimen, NMW2013.39G.28, Edwards *et al*., 2013, fig. 7D, although *Cosmochlaina* is less frequent in dispersed cuticular assemblages. Specimens with incised forms were interpreted as possible maturation states for both taxa. The disintegration noted within some of the incised areas in the younger fossil (Edwards *et al*., 2013, fig. 8E) is also seen in Figure 11E here.

However there are no exact counterparts in the three dimensionally preserved examples recorded in the Lochkovian, the occasional protuberances finding most similarities with dispersed cuticles of *Cosmochlaina*. By contrast, incised examples with hyphae just below the surface resemble some Devonian intermediate forms (cf. Edwards *et al*., 2013, fig. 8O-Q and detail in fig. 7U, W, Y). Such observations provide further information on fungal thalli in the Silurian, but no advances on the functional significance of the emerging hyphae.

GENERAL DISCUSSION

Our previous studies on thalloid non-embryophyte organisms in the Lower Devonian, which identified the sources of the more ubiquitous dispersed cuticles assigned to *Nematothallus* and *Cosmochlaina* and thus contributed to our understanding of the nature and affinities of early terrestrial vegetation, here have been extended into the Upper Silurian. We have now shown that similarly stratified thalli with tripartite organisation of fungal affinity co-existed with plants of axial construction, be they tracheophytes or cryptophytes (plants of uncertain affinity producing cryptospores (dyads and tetrads)), and thus reinforced Strother's contention that (even allowing for taphonomic biases) such thalloid organisms may have dominated land surfaces in the catchment area of rivers in Late Silurian to Early Devonian times (Strother, 1988). However it seems unlikely that, based on surface characters, the majority of our specimens would have survived as isolated cuticles. Exceptions are those assigned to *Tristatothallus* where the superficial layer (cortex) most closely resembles that in *Nematothallus*. This new taxon lacks the intermediate zone of palisade hyphae seen in the Lower Devonian examples. In addition to those described here, there is a Silurian example from the Upper Ludfordian Roman Camp Beds at Capel Horeb Quarry, south Wales, which shows some poorly preserved palisade hyphae attached

to a *Nematothallus* 'cuticle' (Edwards, 1982, fig. 44). As Edwards hypothesised after describing at least six different types of such 'cuticle' based on patterns of 'units', the parent organisms probably derived from a number of taxa/species although all with thalloid organisation. Since similar 'cuticles' have been isolated from Ordovician (Gray, Massa & Boucot, 1982) and Lower Silurian rocks (Pratt, Phillips & Dennison, 1978; Strother & Traverse, 1979), it is suggested that thalloid fungi and possible lichens, along with cryptophytes (cryptospore-bearing extinct plants, Edwards *et al*., 2014), as evidenced by spores (e.g. Wellman, Osterloff & Mohiuddin, 2003) and algal / cyanobacterial films, were important components of cryptogamic covers /biocrusts in initial phases of terrestrialisation (Edwards *et al*., 2015). There is increasing acceptance in the palaeontological community that cryptogamic covers comprised of a number of lineages that colonised land before tracheophyte-dominated vegetation, as discussed in Edwards *et al*., 2015. A major difference is the lack of a good fossil record of bryophytes, but this niche may well have been occupied by cryptophytes. The fragments here, interpreted as parts of hymenial layers of Ascomycetes, provide some evidence for the affinities of some of the fungi. However, it would be unwise to conclude that all coalified compressions in Lower Devonian and earlier strata had fungal affinities. The most thoroughly investigated examples come from the Lower Silurian Massanutten Sandstones in the Tuscarora Formation (Rhuddanian/Llandovery), Passage Creek, Virginia (e.g. Pratt *et al.,* 1978; Tomescu & Rothwell, 2006). In contrast to our allochthonous material, it was postulated that the organisms lived on the drier habitats in riverine wetlands because many of the compressions possessed pre-burial cracks, reflecting those in underlying substrates, indicative of subaerial exposure. The compressions, which sometimes covered bedding planes, were mostly in the form of isodiametric patches up to 10cm wide usually with irregular outlines, with those with smooth curves possibly representing

the original margins of the organisms. Strap shaped examples were rarer. There was some evidence of penetration of the sediment.

Tomescu and Rothwell (2006) demonstrated stratified organism in five types of compression whose distinction was made partially on thickness and density of layers (based on shades of brown colourisation), degree of folding and composition (filaments vs laminae). The filamentous forms received more detailed attention (Tomescu, Rothwell & Honegger, 2006; Tomescu, Rothwell & Honegger, 2009a) revealing trichomes consisting of ensheathed spherical cells embedded in an amorphous matrix. Based on dimensions and organisation, the organisms were considered oscillatorian cyanophytes and named *Pratella massanuttense* (Tomescu *et al.*, 2009a). The authors had also recognised rod shaped bacteria in the amorphous matrix surrounding the filaments (Tomescu, Rothwell & Honegger, 2008). Further potential diversity in composition was investigated using an experimental approach based on comparative survival of lineages during fossilisation (Tomescu et al., 2010). Substrates included cyanobacteria, red, brown and green algae, lichens and bryophytes. Representatives of all groups survived procedures involving compressions of material between wetted paper at 130ºC, although degree of cellular preservation varied among and within lineages. Thus for example, tissues of the brown alga *Fucus*, the red alga *Mazzaella* and the lichen, *Parmotrema* remained pristine, but cellular construction had disappeared in green algal *Spirogyra*, ascocarps and basidiocarps of fungi, and gametophytic thalli of bryophytes, *Marchantia* and *Anthoceros*. Such experiments indicate the fossilisation potential of the various lineages, but are not particularly helpful in identifying the nature of the Silurian compressions. A more direct approach involved sampling of the carbon isotopic values of the forms themselves, and comparing those with values for extant

lineages, taking into account contemporary values for atmospheric CO_2 (Tomescu *et al*., 2009b). They found that values from organic matter recovered from Ordovician and Silurian rocks in the Appalachians (including those from the Tuscarora Formation) compared favourably from those of extant liverworts, with the inference that some of the compressions might derive from liverworts, although today their fractionation values can overlap those of fresh water algae. The possibility that the compressions might have had a gametophytic origin, although in this case from basal tracheophytes as well as bryophytes, has also been suggested by Strother (2010).

It would clearly be impractical to investigate ALL coalified compressions preserved in Ordovician to Lower Devonian rocks to the same depth as adopted by Tomescu and colleagues, Strother and the group in Cardiff. However all such 'black patches' and their frequencies should at least be recorded. We have argued that the somewhat limited direct evidence they provide plus an understanding of the composition and distribution of cryptogamic covers today, allows insights into the nature of early land vegetation before the domination of tracheophytes. In Upper Silurian and Devonian palaeobotanical studies the latter have 'stolen the show', yet this must have been a period of competition between the vascular and non-vascular cryptogams. We know from the spore record that the cryptospore-producing cryptophytes (basal embryophytes) disappeared in the Lower Devonian and that the same source is not helpful in tracing the early history of bryophytes, but what happened to the lichenised and non-lichenized fungi? *Prototaxites* is recorded from the Silurian to the Upper Devonian (Famennian) (Arnold, 1952). The time has surely come to turn attention to the 'black patches' throughout this time interval.

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FIGURE LEGENDS

Figure 1. A. Overall tripartite structure in vertical section, with upper cortex (a), intermediate layer of wide hyphae (b) and a basal layer of narrow hyphae (c). B. Vertical fracture through *Tristratothallus*, showing intermediate layer of interweaving wide hyphae. C. Vertical fracture through of *Nematothallus*, showing intermediate layer of wide hyphae running perpendicular to the cortex, referred to here as palisade. D. Three-dimensional reconstruction of *Tristratothallus*, illustrating the orientation of the thallus. Orientation is interpreted, as extracted fragments are not *in situ*, based on: i) the presence of the cuticle, its composition and presence of perforations suggests exposure to the atmosphere; ii) the zone of hyphae would seem an adaptation to attachment to substrate for support and absorption.

Figure 2. *Tristratothallus ludfordensis* gen. et sp. nov., Ludford Lane (LL), Ludlow, Upper Silurian. Scale bars: A-E = 50µm; F-H = 20µm. A = Vertical fracture (VF) through 3-layered thallus; a = cortex, b = intervening ± homogenised, c = basal hyphae. Specimen LL(L)07/13. B = VF through thallus of holotype, specimen LL(L)07/03. a = cortex, b = intervening ± homogenised, c = basal hyphae. C & D = Oblique tangential fracture through thallus. Specimens LL(L)07/06 & LL(L)07/17 respectively. E = Oblique fracture showing details of cortex and outer region of intervening layer. Arrows indicate minute perforations. Holotype specimen LL(L)07/03. F = Surface of holotype cortex. G & H = Fractured contact between cortex and tissue beneath. G, holotype; H, LL(L)07/13.

Figure 3. *Tristratothallus ludfordensis* gen. et sp. nov., Ludford Lane (LL), Ludlow,

Upper Silurian. Scale bars: A, B, I-K = 50μm; C-H, L-O = 20μm; P = 10μm; Q = 200μm. A-C = Details of cortex. A, ± intact. LL(L)07/13. B, cortical cells with outer periclinal wall removed. Holotype. C, anticlinal walls revealed. LL(L)0 7/13. D-G = Detail of perforations in exposed walls intervening tissue, D = holotype. E, F = LL(L)07/17, G = LL(L)07/06. H-K = Hyphae in basal zone. H = holotype, I = LL(L)07/06, J = LL(L)07/17, K = LL(L)07/13. L-P = Aspects of fractured hyphal wefts. L = holotype, M-P = LL(L)07/17. Q = Protruding hyphae from intact thallus. LL(L)07/13.

Figure 4. cf. *T. ludfordensis*, Ludford Lane (LL), Ludlow, Upper Silurian. Specimen LL(L)06/16. Scale bars = 20μm, except A = 200μm. A = Lower surface of fragment, before investigation. B, E-G = Edge of specimen with cortex, and remnants of underlying tissues. Arrows in E indicate fragments of hyphae. C, D = Superficial view of cortex, with perforated cells in C. H = Projecting cortical cells and remnants of underlying tissues with perforations. I = Fractured cortex lacking inner periclinal walls. J = Vertically fractured thallus.

Figure 5. *Nematothallus* sp. Ludford Lane (LL), Ludlow, Upper Silurian. Specimens LL(L) 4/02, LL(L)04/13, LL(L)07/02, LL(L)16/05. Scale bars: A = 200μm; B, D, E, F, H = 50μm; C = 100μm; G = 20 μm. A = Lower surface of specimen LL(L)04/13. Fragment with tripartite organisation. B = Fractured thallus. Upper surface bottom left. a = cortex, b = palisade, c = fused basal layer. LL(L)16/05. C = Enlargement of most complete specimen (A), with trilayered tissues viewed from below. D = Fractured fragment with imperfectly preserved tripartite organisation. LL(L)07/02. E = Internal view of remains of cortex resembling that of *Nematothallus,* with fragments of internal tissues. LL(L)07/02. F = Fractured section showing palisade tissue and

fragmentary basal hyphal zone. LL(L)16/05. G = Superficial view of basal zone with fragments of hyphae. LL(L)16/05. H = Fragment with three zones imperfectly preserved. LL(L)07/02.

Figure 6. Ascomycete apothecial fragment. Ludford Lane (LL), Ludlow, Upper Silurian. Specimen LL(L)03/02. Scale bars: B, F, H, I = 20µm; E, G = 50µm; A, C, D = 100µm. A = Fragment before study. B = Laminal view of the hymenial surface layer. C = Fragment viewed from below, showing interweaving hyphae on lower surface and hymenial layer, built up by paraphyses and asci. Arrow indicates a longitudinally fractured, polysporic ascus. D = Ascus with remains of spores (arrows). Hymenial surface at top of field. E = Thick arrows indicate longitudinally fractured, polysporic asci between paraphyses. Thin arrow points to the thin margin of the apothecium. F, G = Interwoven basal hyphae. H, I = Junction between hymenial surface (cortex) and hymenial layers.

Figure 7. Putative apothecial fragment. Ludford Lane (LL), Ludlow, Upper Silurian. Specimen LL(L)16/02. Scale bars: A, E, G = 50µm, B, C, D, F = 20µm. A = Fractured surface viewed from below with presumed hymenial layer (palisade) overlying homogenised basal zone. B = Voids in presumed hymenial layer putatively once occupied by asci. Note junction between epihymenial (cortex) and hymenial layer obscured by disorganised material. C = Oblique view of homogenised hymenial surface layer. D = Fractured anticlinal walls of hymenial surface. E = Reticulate appearance of deeper seated layer. F = Fractured section of fused basal hyphae. G = Surface of basal zone with possible contaminant hyphae.

Figure 8. Putative apothecial fragment. Ludford Lane (LL), Ludlow, Upper Silurian.

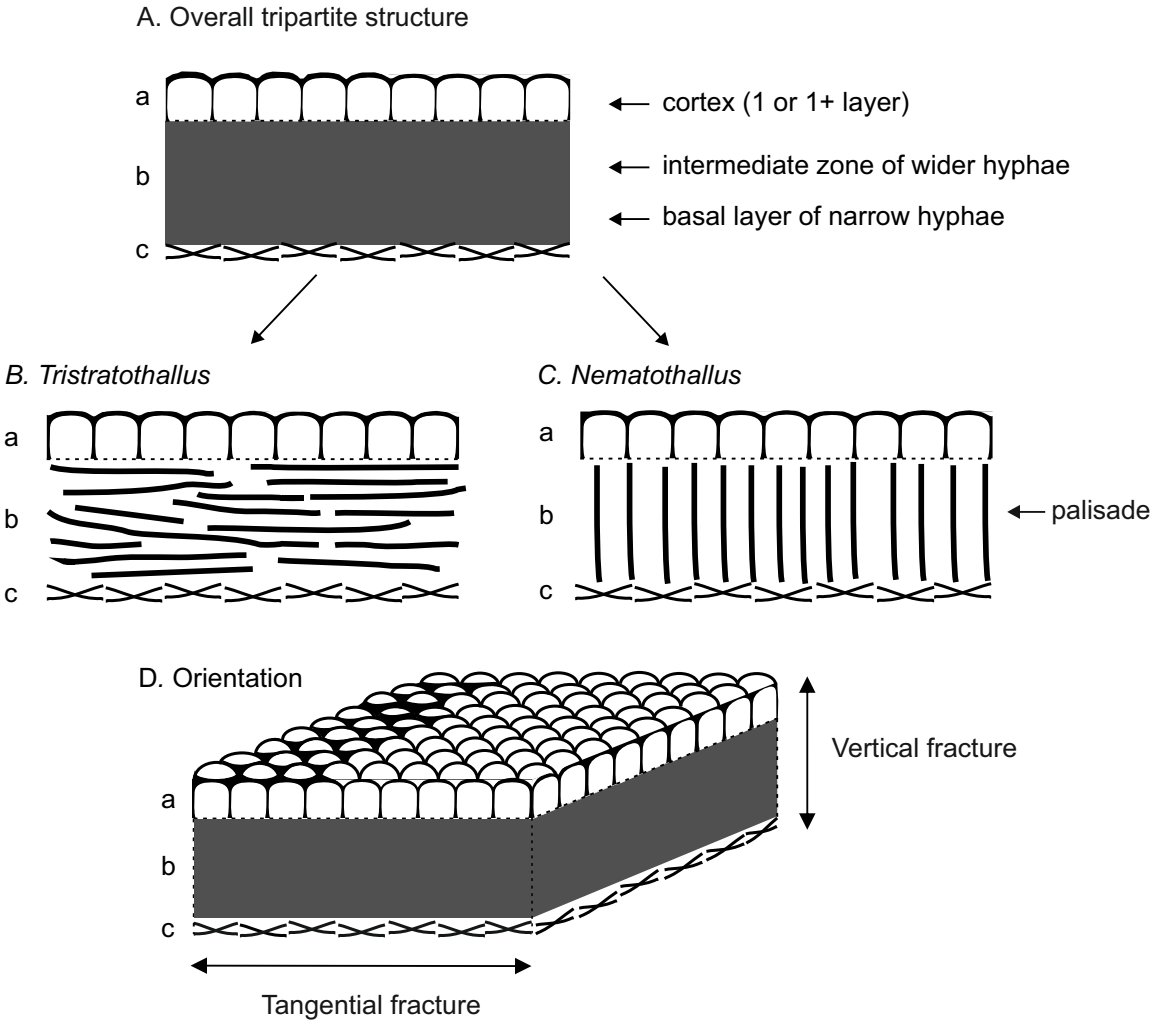
Specimen LL(L)05/09. Scale bars: A-D = 50μm; E-H = 20μm. A = Bilayered fragment: basal hyphal zone absent. Note prominent thick walled hyphae. B = Oblique view of junction between presumed hymenial surface layer (epihymenium; note ridges on inner surface of tip cells) and disintegrating hyphal complexes below surface of hymenium. D = Remains of presumed paraphyses. Arrow indicates putative polysporic ascus. Junction between epihymenial and hymenial layer indecipherable. E = Image in C magnified and tilted, revealing hollow tip cell. F = Remains of thinner walled hyphae in hymenial layer. G = Arrowed are possible ends of thick walled hyphae. H = Junction between epihymenial and hymenial layer. * = thick walled hyphae with intact tip.

Figure 9. cf. *Cosmochlaina.* Ludford Lane (LL), Ludlow, Upper Silurian. Specimen LL(L)10/21. Scale bars: A, B, M = 200μm; C-L, N, O = 20μm; P = 10μm. A, B = Lower and upper surfaces of tripartite thallus. Arrows in A indicate masses of small tubes (?medullary spots). C-L = Variation in appearance of outer surface of cortex. C = typical of *Nematothallus*. D, E = areas with and without discrete cortical cells. Arrow on E indicates small projection. F, G = irregular cortical cells with occasional protruding hyphae (arrows). H = cortical cells with extended outer walls. I = putative protruding ?septate hypha. J = decomposing central area of cortical cell. K = protruding hypha. L = cortical cells absent in lower part exposing disorganised tissue below. M = Blocks of palisade tissue, but individual hyphae not distinct. N = Areas in palisade zone possibly comprising fused ascospores. O = Longitudinal fracture of cortex and elongate structures beneath. P = Fused hyphae in basal zone.

Figure 10. cf. *Cosmochlaina.* Ludford Lane, Ludlow, Upper Silurian. Specimens LL(L)04/06, LL(L)11/11. Scale bars: A = 200μm; B, G, I-L = 50μm; C-F = 20μm; H =

100µm. A = Entire fragment with ridged upper surface. LL(L)04/06. B = Edge of fragment with dimpled surface and putative emergent hyphae. LL(L)04/06. C = Surface of B further enlarged D, E = Disintegrating surface, putative emergent hypha arrowed. LL(L)11/11. F, G = Lower surface of fragment in A-C. F = interwoven hyphae. G = cortex, (a) and remains of palisade zone (b). H = Tripartite thallus viewed from below where fused basal hyphae. Upper surface illustrated in D & E. I, J = Part of H magnified, note hyphal tips in obliquely fractured ?cortical cells. K = Emergent hyphae in homogenised cortex. LL(L)11/11. L = Curved edge of specimen LL(L)11/11, emerging hypha arrowed. M = ?Contaminant hyphae on surface of fused basal zone. LL(L)11/11.

Figure 11. Intermediate form (*sensu* Edwards *et al.* 2013), Ludford Lane, Ludlow, Upper Silurian. Specimens LL(L)02/07, LL(L)10/23, LL(L)11/09. Scale bars = 20µm, except in E = 5µm. A = Surface view of specimen with rounded incisions. LL(L)10/23. B = Thallus viewed from below. Note partially inrolled cortex with incisions and remnants of palisade tissue. LL(L)11/09. C = As in B, but distal region of palisade tissue with terminally inflated hyphae. LL(L)02/07. D = Obliquely fractured tripartite thallus viewed from below. Note relatively well preserved hyphae on left. LL(L)02/07. E = Disintegrating outer wall at centre of incision. LL(L)02/07. F = Presumed action bacterial hyphae colonising the thallus. LL(L)11/09.


A. Overall tripartite structure
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intermediate zone of wider hyphae
basal layer of narrow hyphae
B. Tristratothallus
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C. Nematothallus
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palisade
D. Orientation
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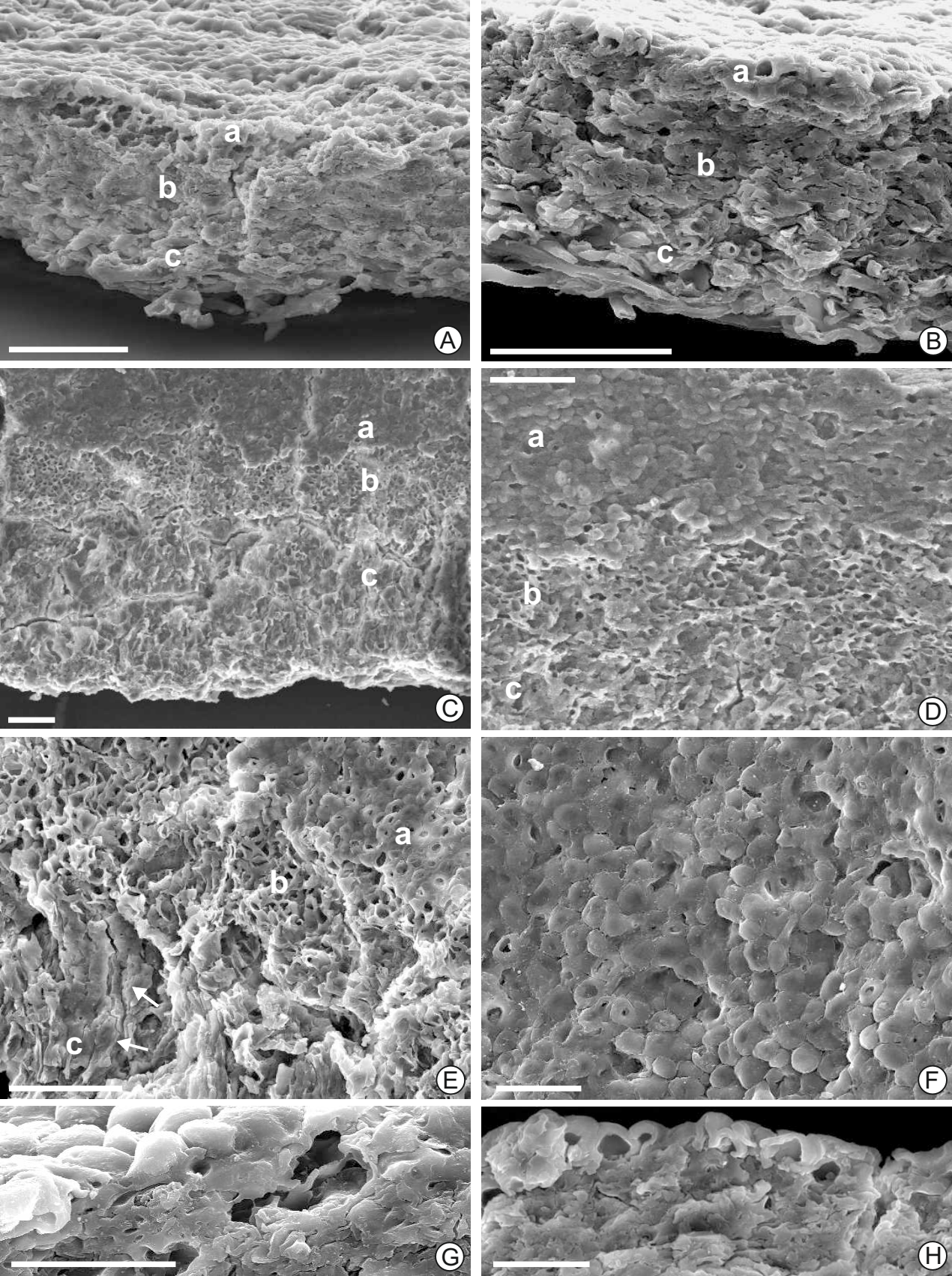
Vertical fracture
Tangential fracture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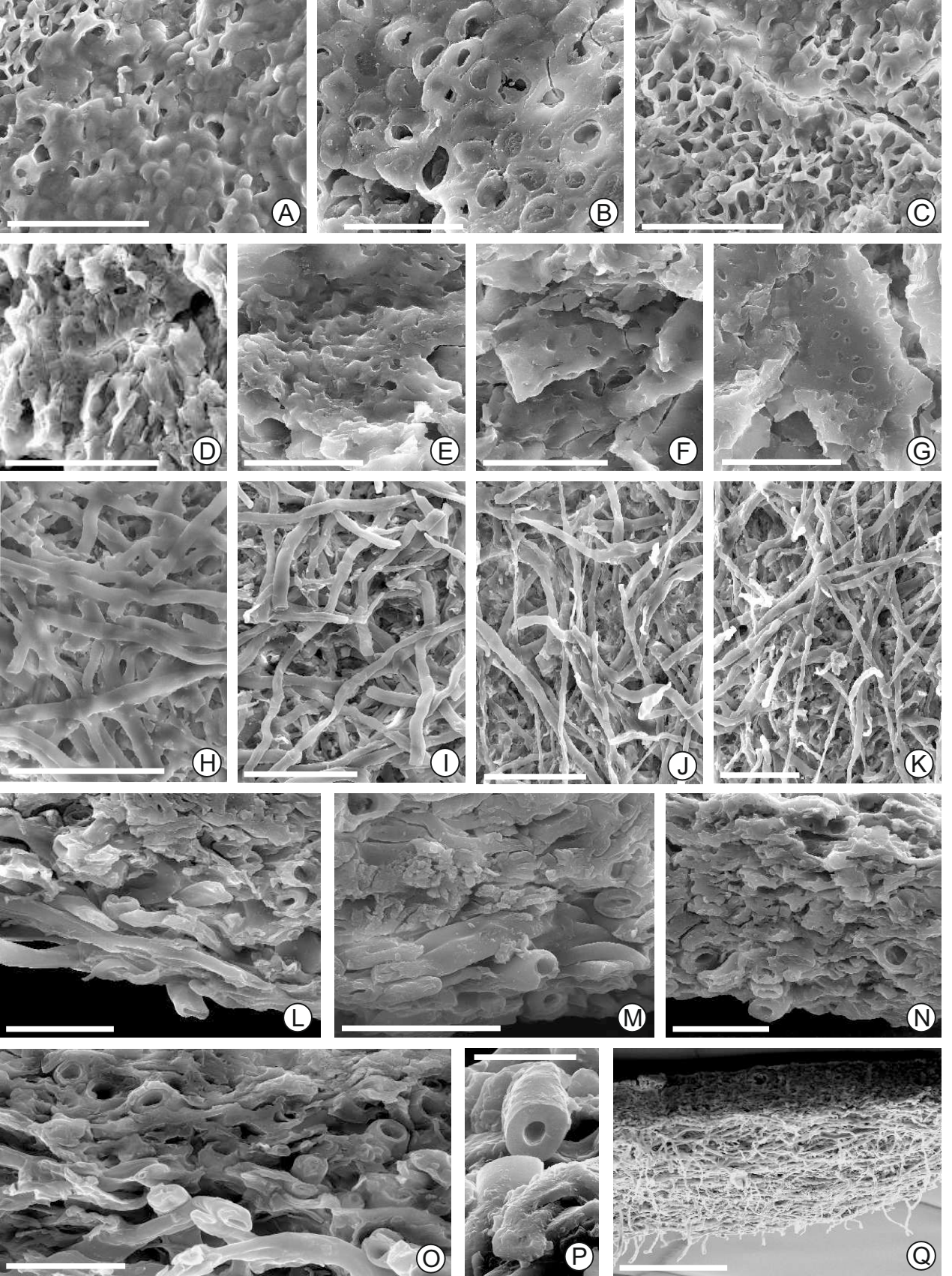
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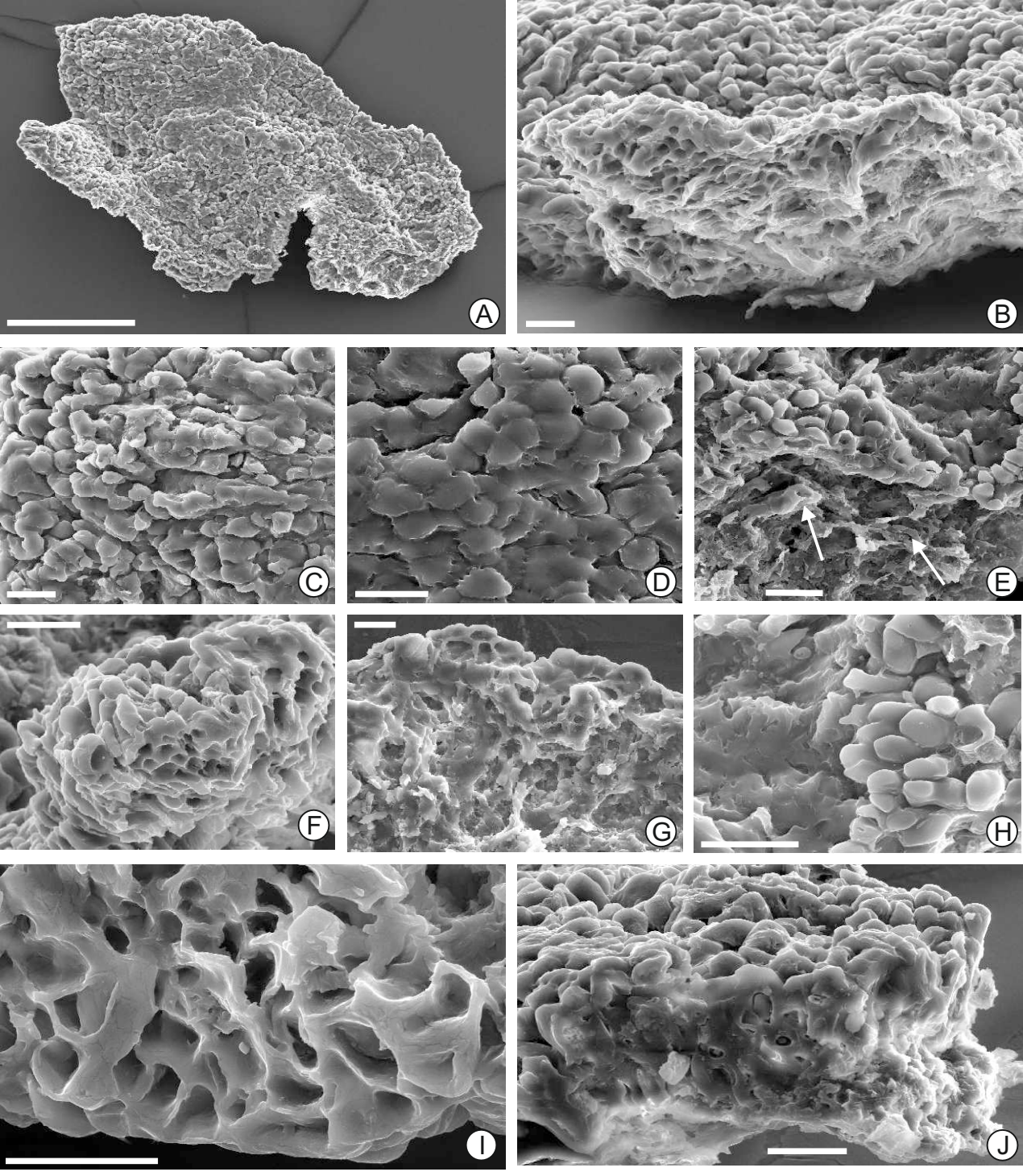
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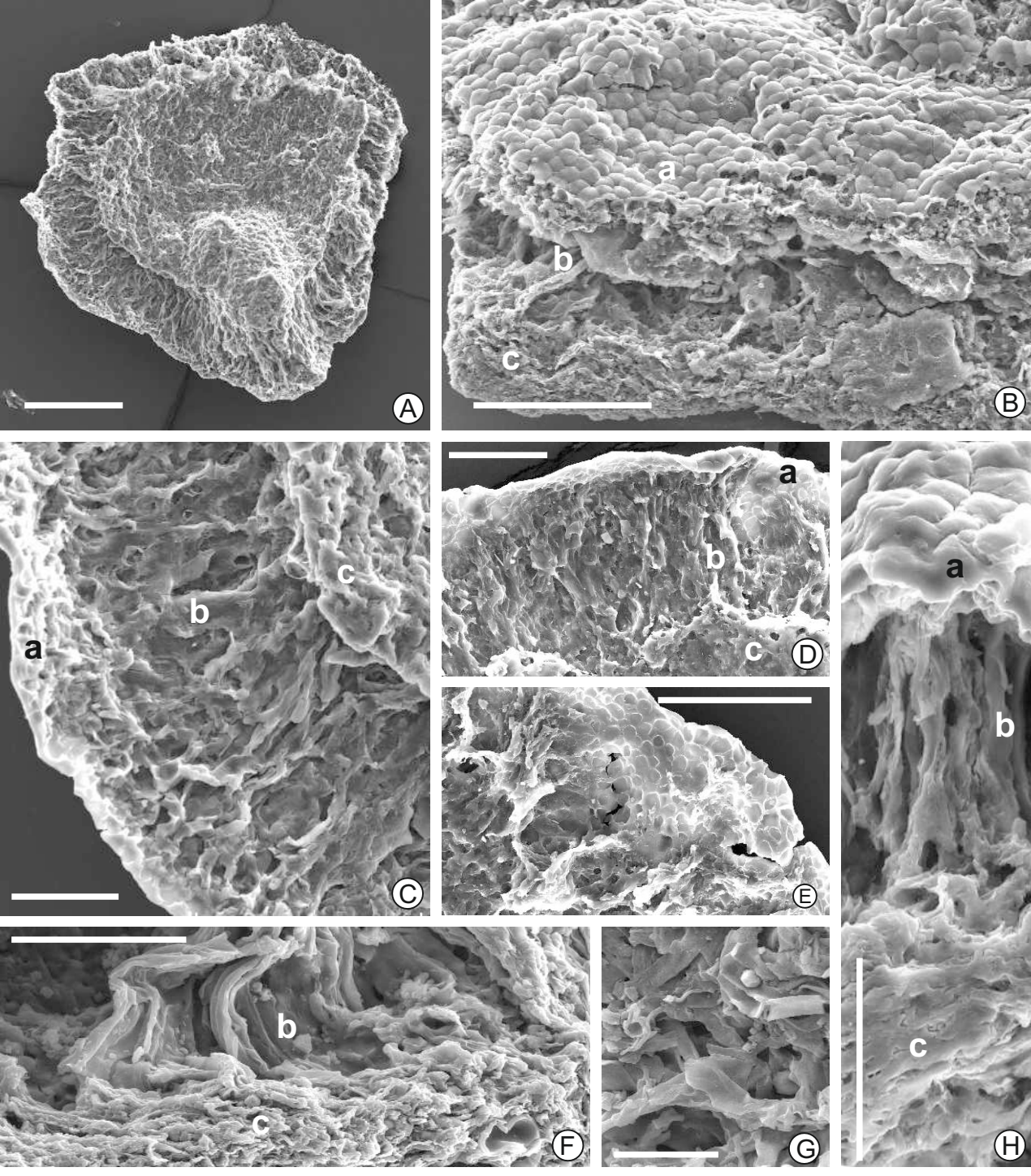
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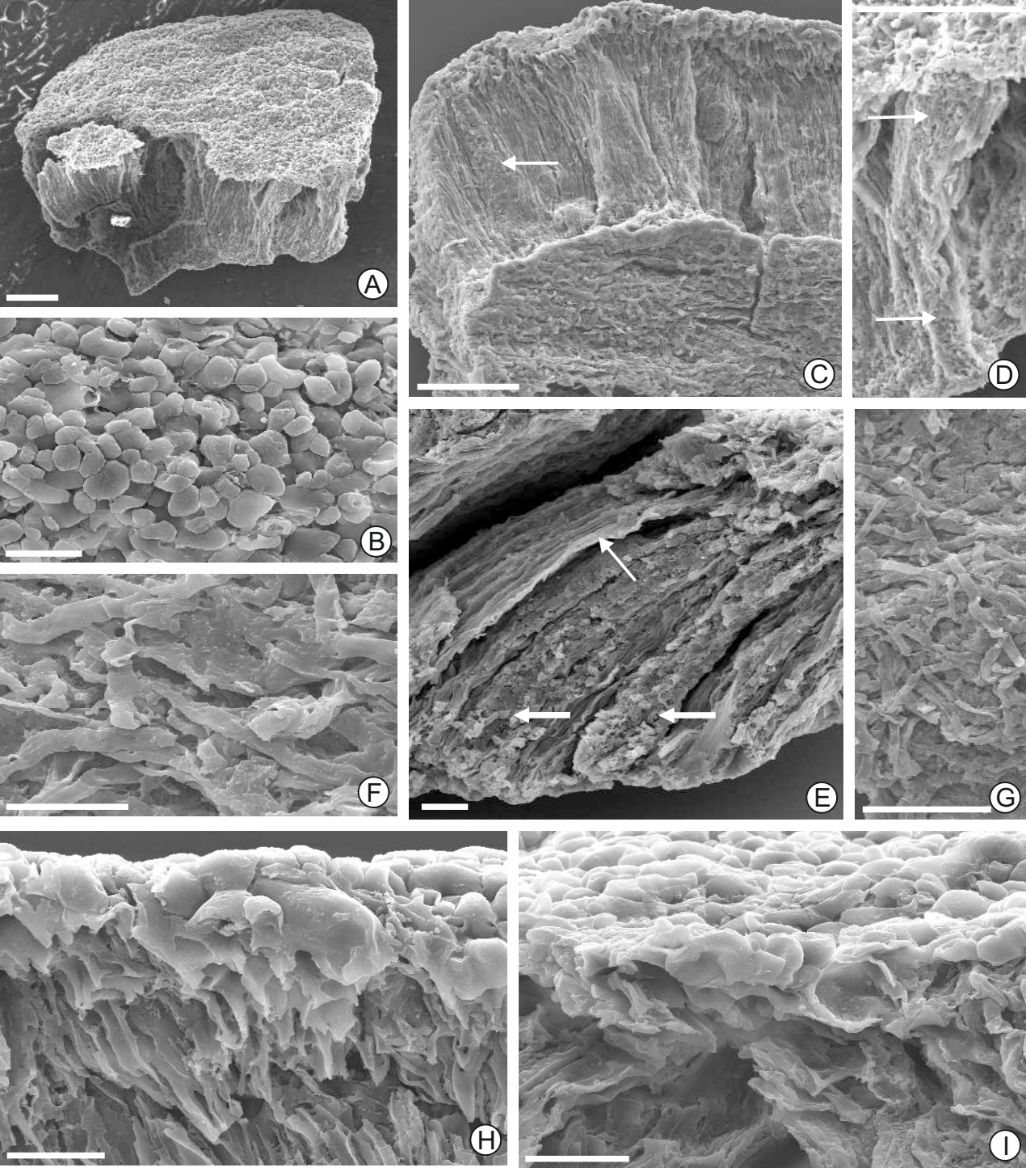
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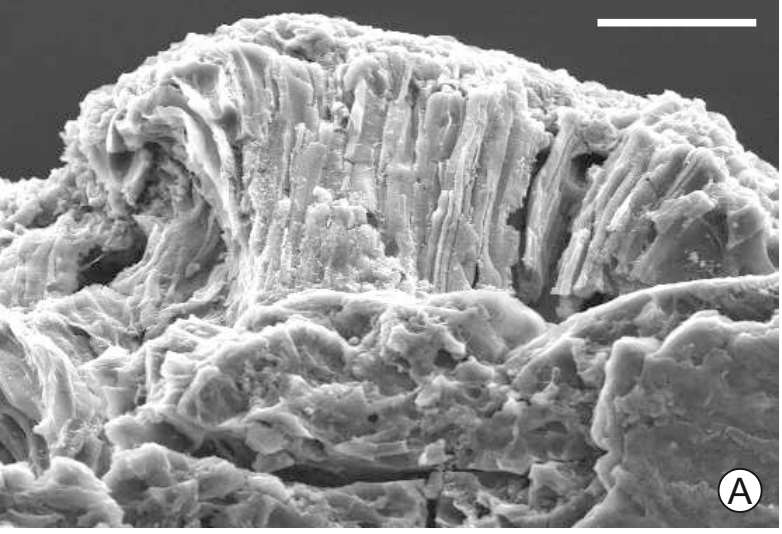
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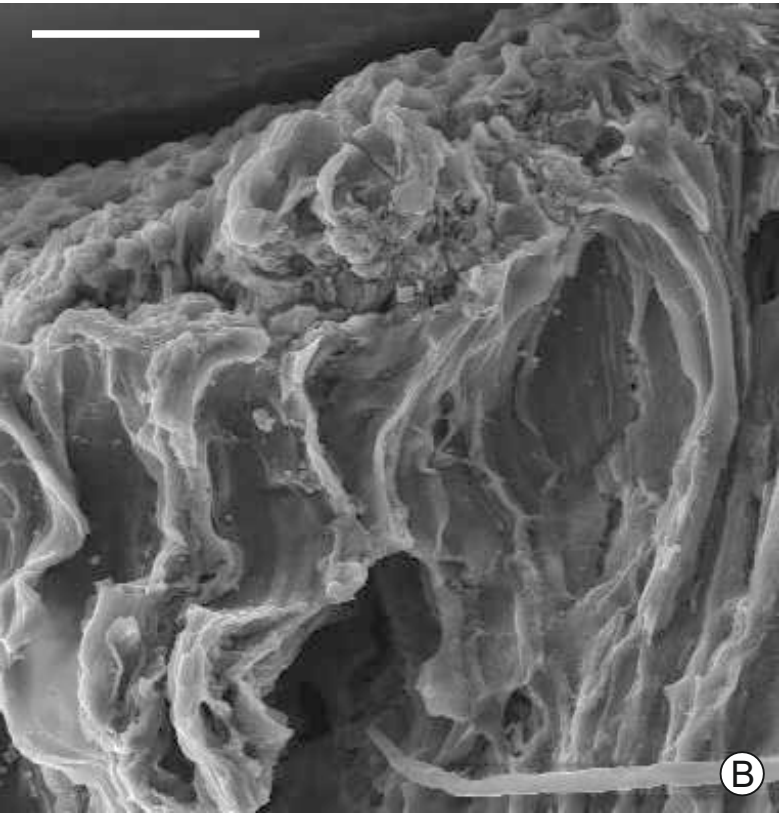
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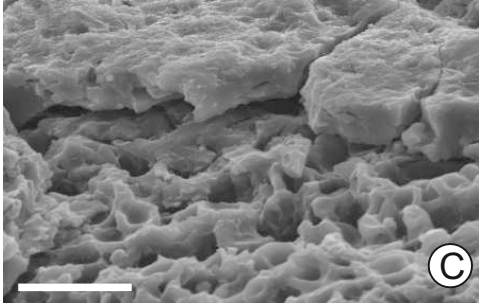
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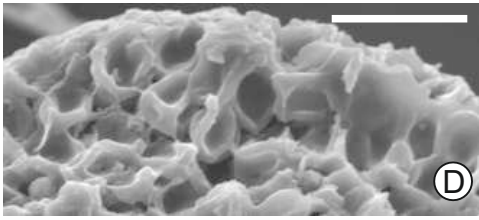
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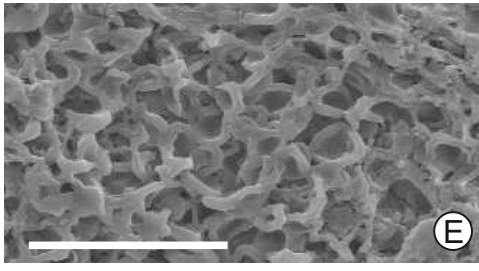
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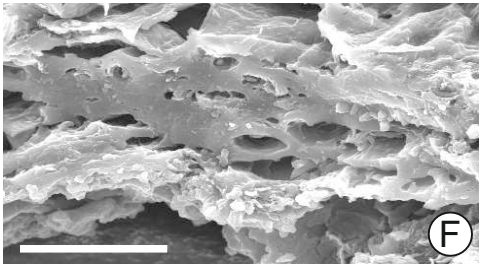
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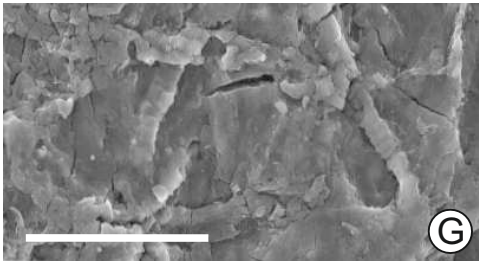
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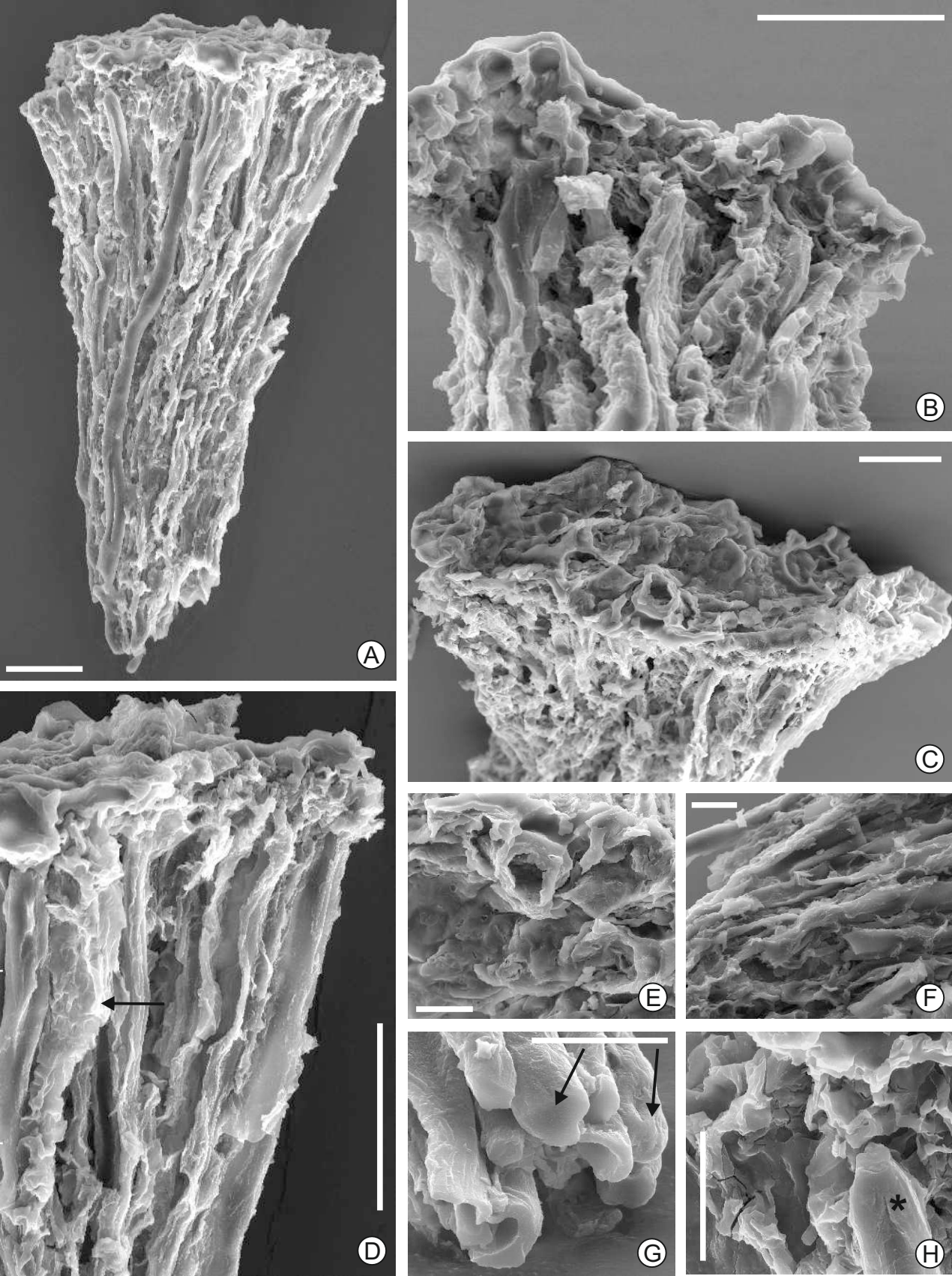
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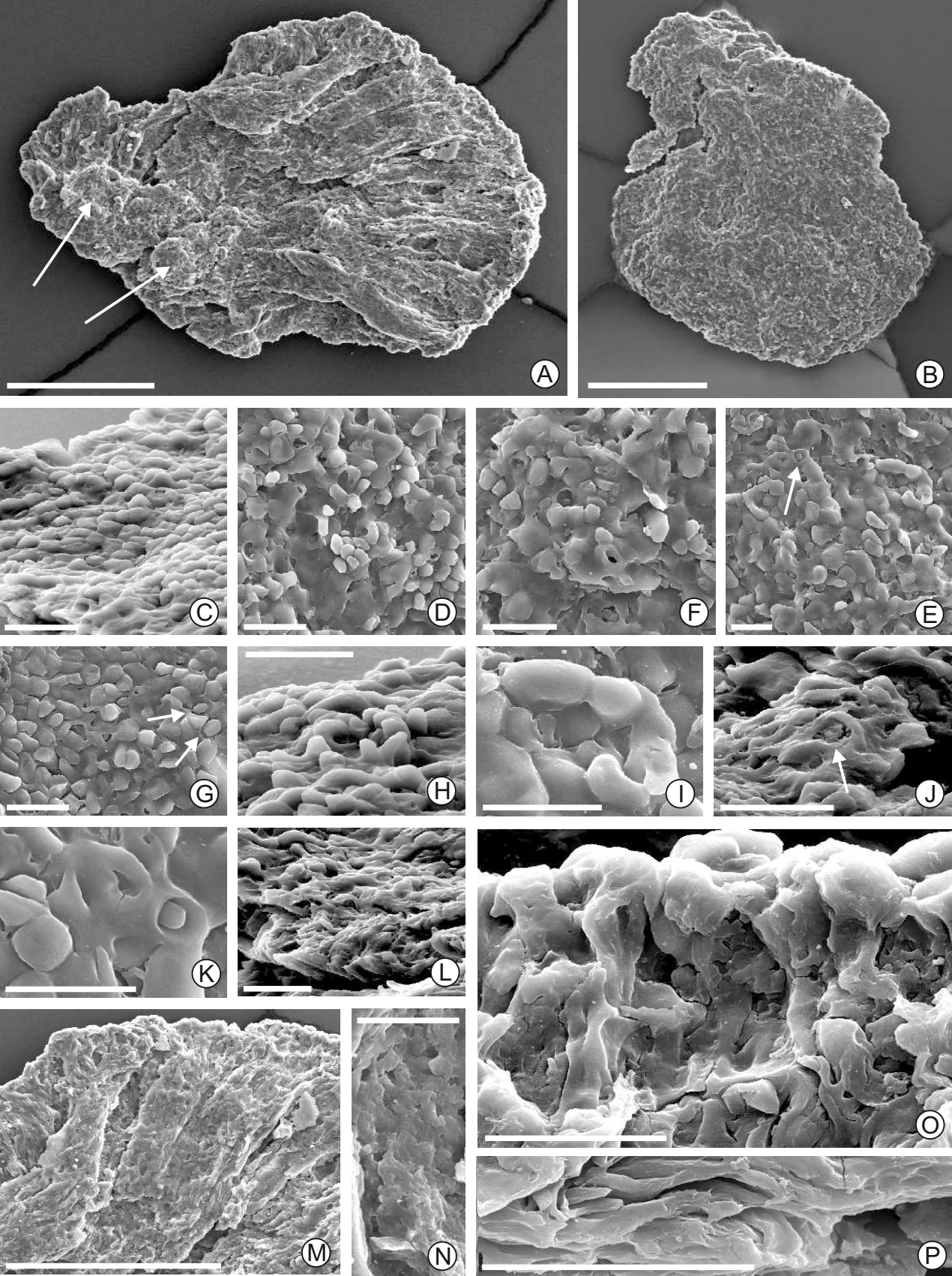
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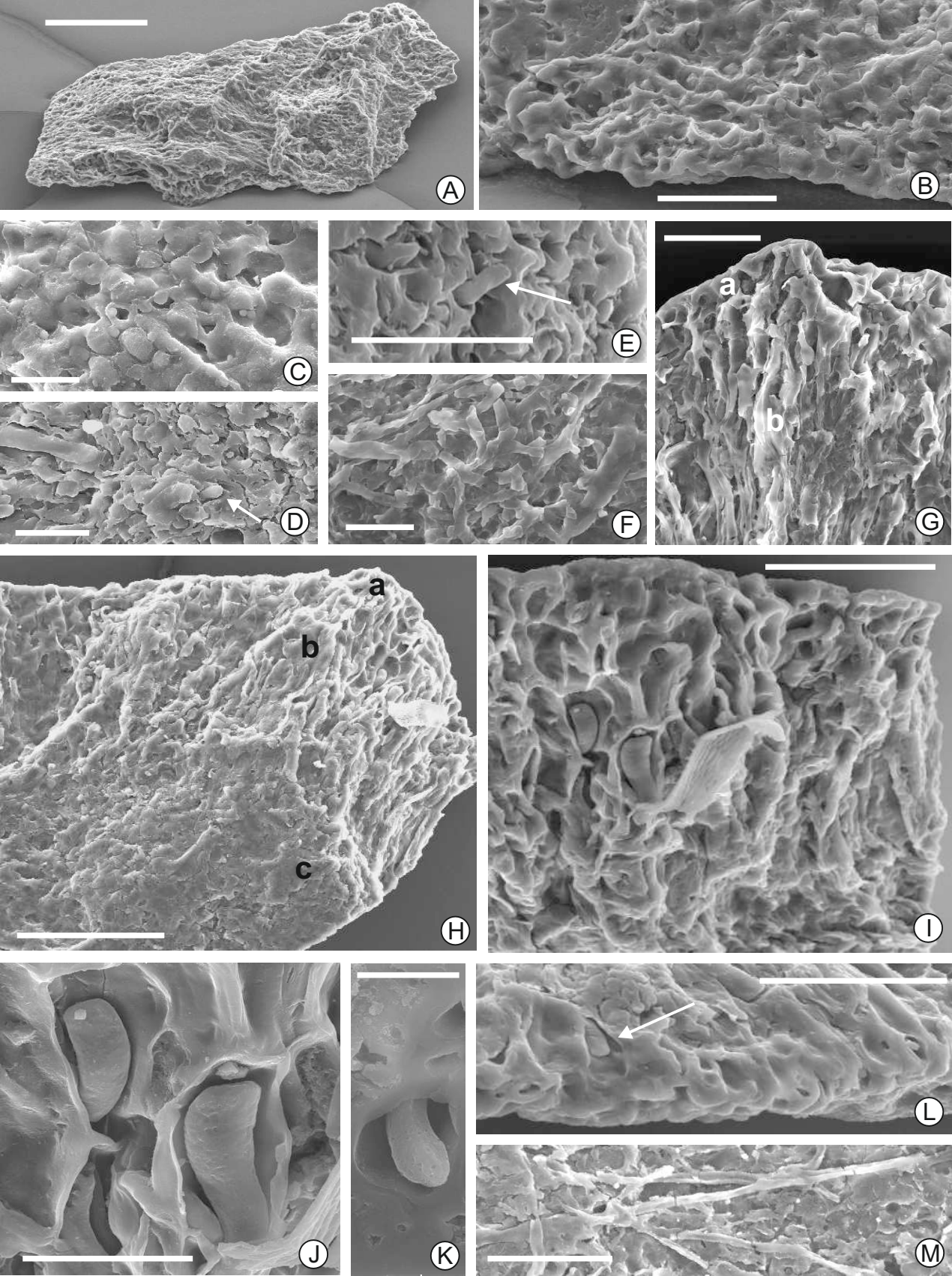
A
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C
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a
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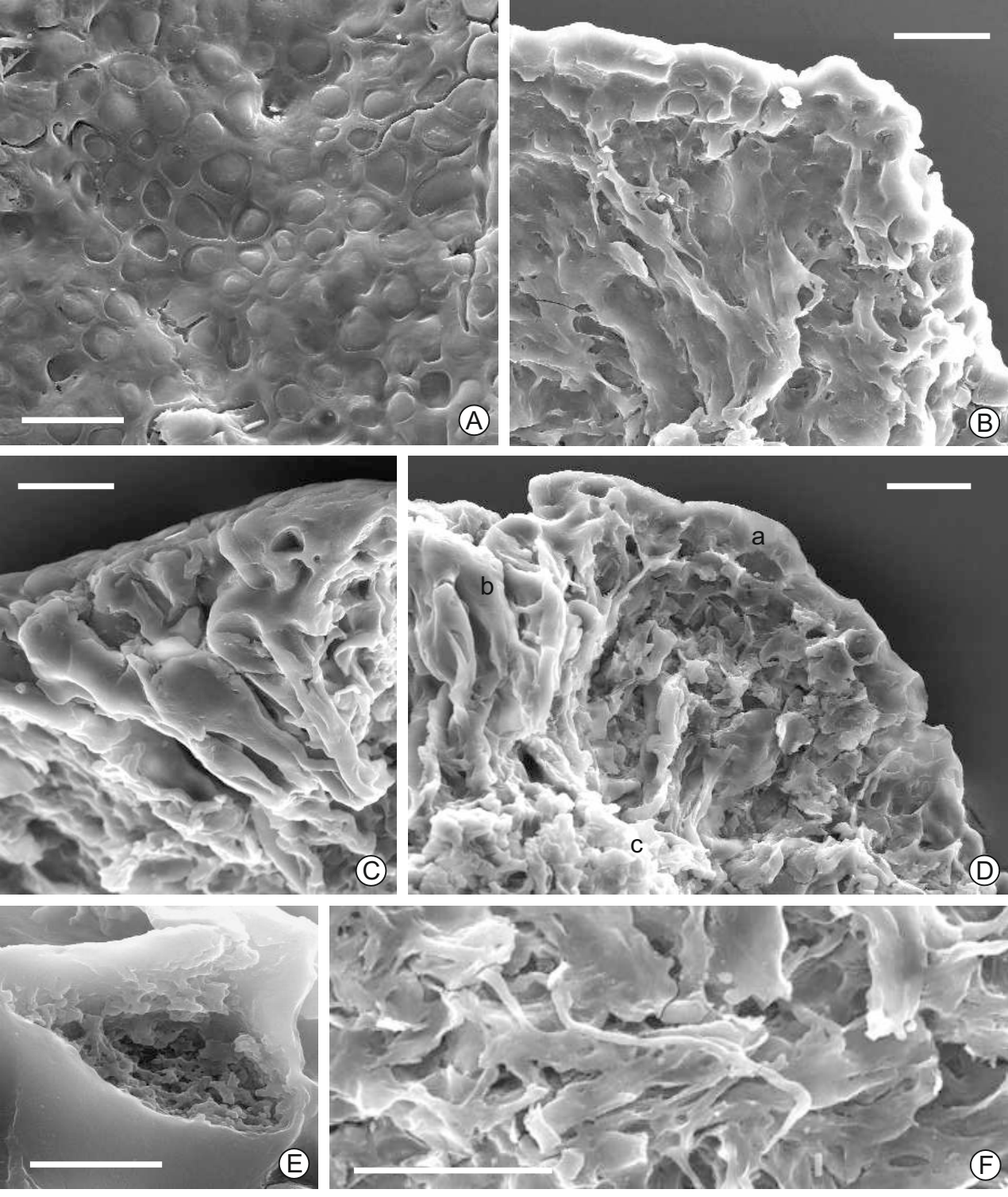
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C
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F